# High-Density Bioconjugated Phospholipid Polymer Brushes for Highly Sensitive Immunoassays

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**Introduction:** 2-Methacryoyloxyethyl phosphorylcholine (MPC) is a bioinspired monomer with phospnorylcholine group. From the MPC, biocompatible polymers that significantly reduce non-specific adsorption of proteins and cell adhesion have been synthesized [1]. We chose a copolymer of MPC with *p*-nitrophenyloxycarbonylethylene glycol methacrylate (MEONP), a monomer with active ester group to react with amino groups of proteins. The aim is to synthesize an antibody-bearing macromolecule, with various geometries, for further applications in immunologic tests like the enzyme linked immunosorbent assay (ELISA) which uses antibodies immobilized on a flat surface and blocking reagents like bovine serum albumin (BSA) or poly(MPC-co-styrene) [2] for preventing the undesired adsorption of targeted proteins onto the solid substrate. In this study, we designed a "tree" molecule composed of MPC and MEONP which role is to enhance the immunogenic reaction by increasing the mobility and accessibility of primary antibodies and, thanks to the phosphorylcholine groups, to prevent biofouling, which decreases the accuracy of this test. For the synthesis, atom transfer radical polymerization (ATRP) has been selected for an easy control of molecule design (Figure 1).


Figure 1. Synthesis of the MPC copolymer by ATRP

**Methods:** A poly(ethylene) (PE) sheet, previously coated with poly(styrene-co-chloromethylstyrene) (PStCMS) with various compositions of chloromethylstyrene (10, 20 and 50%) in dichloroethane (PStCMS10, 20 and 50), was made to react with CuCl, $CuBr_2$, 2,2'-bipyridyl, MPC and MEONP (at the same time for copolymers) in ethanol [3]. Block copolymers were synthesized by a first reaction with MPC only on a PStCMS20-coated surface and after washing the film, by a subsequent reaction with MEONP in the same conditions for a shorter time (MEONP capping). The surface was then characterized by X-ray photoelectron spectroscopy (XPS), ellipsometry and atomic force microscopy (AFM). Anti-human thyroid stimulating hormone (TSH) antibodies in buffer (pH=8, concentration of 10µg/mL) were subsequently made to react with these films for 20h and observed by AFM after washing with PBS. These films immobilized with these antibodies were also tested with a TSH sandwich enzyme-linked immunosorbent assay (ELISA).

**Results/Discussion:** Ellipsometry, XPS and AFM results showed the successful grafting of MPC and MEONP on a 250-nm-thick layer of PStCMS, which is melted into the PET sheet and is thus strongly immobilized. The MPC polymer layer is 50 to 60 nm thick. NMR data showed a very low MEONP composition in random copolymers which is between 1 and 5%. According to AFM observation, smooth and very homogeneous surfaces were obtained with a mean square roughness between 2 and 3nm (Figure 2-a). Antibodies were covalently immobilized on the surfaces with a good density (Figure 2-b) in packs of 40 to 500 units. The surface coated with PStCMS20 and grafted with poly (MPC-co-MEONP) was tested by an ELISA test along with two other blocking reagents (Figure 3). The results show an increase of 80% in the signal/noise ratio for this surface compared to a surface blocked with BSA. The surface grafted with the block copolymer was also tested and displayed a signal/noise ratio four times higher than for the random-copolymer-grafted surface on the condition that it is treated with aminoethanol for deactivating of active ester sites after antibody immobilization.

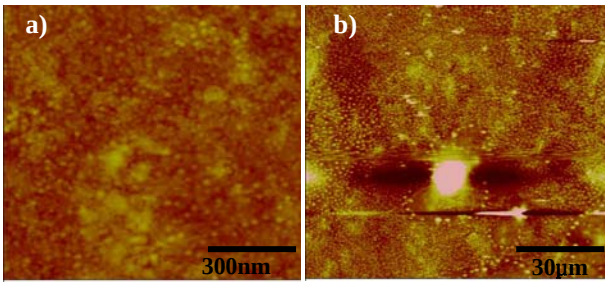


Figure 2. AFM observation
a) Surface grafted with poly(MPC-block-MEONP)
b) Same as a) with immobilized antibodies

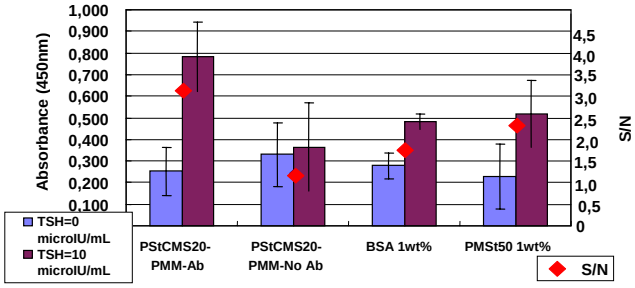


Figure 3. Results of the ELISA test
a) Surface initiated with PStCMS20 and grafted with poly(MPC-co-MEONP) with antibodies
b) Same as a) without antibodies
c) Plastic substrate with antibodies blocked with BSA
d) Same as c) but blocked with poly(MPC-co-Styrene) (50% of MPC in composition)

**Conclusions:** Poly(MPC-co-MEONP), which can react with antibodies, was successfully grafted onto a plastic substrate and smooth and homogeneous surfaces were obtained. The surface grafted with poly(MPC-co-MEONP) displayed a great increase in the sensitivity of the TSH sandwich ELISA test. Characteristics like the blocking and antibody immobilization properties are still to be studied and improved.

**References:**

[1] K. Ishihara Sci. Tech. Adv. Mater. 2000;1: 131-138
[2] S. Sakaki Polym. J. 2000; 32: 637-641
[3] Z. Cheng Ind. Eng. Chem. Res. 2005; 44: 7098-7104